

TI Cytotoxicity of **taxol** in vitro against human and rat malignant brain tumors  
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 DN 121:169943  
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 AU Cahan, Mitchell A.; Walter, Kevin A.; Colvin, O. Michael; Brem, Henry  
 CS Department Neurological Surgery, Johns Hopkins University School Medicine, Baltimore, MD, USA  
 SO Cancer Chemotherapy and Pharmacology (1994), 33(5), 441-4  
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 DT Journal  
 LA English  
 AB **Taxol** is a novel antitumor alkaloid that has shown clin. activity against several tumors, including ovarian and **breast carcinoma** and melanoma. To evaluate **taxol**'s potential as a therapy for malignant brain tumors, we measured the sensitivity of four human (U87, U373, H80, and D324) and two rat (9L, F98) brain-tumor cell lines to **taxol**. The cells were exposed to **taxol** in vitro using a clonogenic assay. Log cell kill (LD90) occurred at concns. of 42 (9L), 25 (F98), 19 (H80), 7.2 (U373), 9.1 (U87), and 3.9 nM (D324) when cells were continuously exposed to **taxol** for 6-8 days. The human cell lines were uniformly more sensitive to **taxol** than were the rat lines. The duration of exposure had a significant effect on **taxol**'s cytotoxicity. When cells were exposed to **taxol** for 1 h the LD90 increased to 890 nM for the 9L rat line and 280 nM for the human U373 line. On the basis of these results, we conclude that **taxol** has significant potency in vitro against malignant brain tumors and that the activity occurs at concns. of **taxol** that have previously been shown to be effective for several tumors against which the drug is currently being evaluated clin.

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ST **taxol** malignant brain tumor  
 IT Neoplasm inhibitors  
 (brain, cytotoxicity of **taxol** against human and rat malignant brain tumors)  
 IT Brain, neoplasm  
 (inhibitors, cytotoxicity of **taxol** against human and rat malignant brain tumors)  
 IT 33069-62-4, **Taxol**  
 RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)  
 (cytotoxicity of **taxol** against human and rat malignant brain tumors)

replacing sodium methoxide solution with reactive amounts of alkaline metals or inorganic salts such as Na.sup.2+, . . . Cs.sup.2-, imidazole, morpholine, piperazine, piperidine, pyrazole, pyridine, adenosine, cinchonine, glucosamine, quinine, quinidine, tetracycline, or verapamil resulting in salt forms of **combretastatin A -4P** with varying solubility.

L2 ANSWER 2 OF 2 USPATFULL  
TI Tubulin binding ligands and corresponding prodrug constructs  
AN 2002:106432 USPATFULL  
TI Tubulin binding ligands and corresponding prodrug constructs  
IN Pinney, Kevin G., Hewitt, TX, UNITED STATES  
Mocharla, Vani P., Waco, TX, UNITED STATES  
Chen, Zhi, Hamden, CT, UNITED STATES  
Garner, Charles M., McGregor, TX, UNITED STATES  
Ghatak, Anjan, Waco, TX, UNITED STATES  
Hadimani, Mallinath, Waco, TX, UNITED STATES  
Kessler, Jimmy, Waco, TX, UNITED STATES  
Dorsey, James M., Waco, TX, UNITED STATES  
PI US 2002055643 A1 20020509  
AI US 2001-804280 A1 20010312 (9)  
PRAI US 2000-188295P 20000310 (60)  
DT Utility  
FS APPLICATION  
LREP Daniel S. Hodgins, JACKSON WALKER L.L.P., Suite 2100, 112 E. Pecan Street, San Antonio, TX, 78205  
CLMN Number of Claims: 79  
ECL Exemplary Claim: 1  
DRWN 23 Drawing Page(s)  
LN.CNT 1875  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
AB A diverse set of tubulin binding ligands have been discovered which are structurally characterized, in a general sense, by a semi-rigid molecular framework capable of maintaining aryl-aryl, pseudo pi stacking distances appropriate for molecular recognition of tubulin. In phenolic or amino form, these ligands may be further functionalized to prepare phosphate esters, phosphate salts, and phosphoramidates capable of demonstrating selective targeting and destruction of tumor cell vasculature.  
DETD . . . remarkable activity in terms of tumor growth control in the skid mouse which is comparable to the activity demonstrated by **combretastatin A-4P** (CA-4P) which is currently in human clinical trials. It is important to note that this particular experiment shows only data. . .

=> s l1 and taxol  
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L3 0 L1 AND TAXOL

=> s l2 and taxol  
L4 1 L2 AND TAXOL .

=> d 14 ti, bib, ab, kwic

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SUMM . . . most attractive therapeutic targets in new drug design for the treatment of solid tumors. The heralded success of vincristine and **taxol** along with the promise of combretastatin A-4 (CSA-4) prodrug and dolastatin 10, to name just a few, have firmly established.

SUMM . . . the most recognized and clinically useful members of this class of antimitotic, antitumor agents are vinblastine and vincristine.<sup>3</sup> along with **taxol**.<sup>4</sup> Additionally, the natural products rhizoxin,<sup>5</sup> combretastatin A-4 and A-2,<sup>6</sup> curacin A,<sup>1</sup> podophyllotoxin,<sup>7</sup> epothilones A and B,<sup>8</sup> dolastatin 10,<sup>9</sup> and welwistatin.<sup>10</sup> . . . several key binding sites on tubulin: colchicine site, vinca alkaloid site, and a site on the polymerized

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 and welwistatin.<sup>10</sup> . . . several key binding sites on tubulin:  
 colchicine site, vinca alkaloid site, and a site on the polymerized  
 microtubule to which **taxol** binds.<sup>1a,14</sup>  
 DETD . . . remarkable activity in terms of tumor growth control in the  
 skid mouse which is comparable to the activity demonstrated by  
**combretastatin A-4P** (CA-4P) which is  
 currently in human clinical trials. It is important to note that this  
 particular experiment shows only data. . .  
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 Chemistry of **Taxol**, a Clinically Useful Anticancer Agent, J.  
 Nat. Prod. 1990, 53, 1.  
 DETD [0179] 6. Schiff, P. B.; Fant, J.; Horwitz, S. B., Promotion of  
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 DETD [0180] 7. Swindell, C. S.; Krauss, N. E.; Horwitz, S. B.; Ringel, I.,  
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 Chain Substituents and Variable C-2' Configurations, J. Med. Chem. 1991,  
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 DETD [0201] 29. Rao, S.; Horwitz, S. B.; Ringel, I., Direct Photoaffinity  
 Labeling of Tubulin with **Taxol**, J. Natl. Cancer Inst., 1992,

84, 785.

=>

L2 ANSWER 1 OF 2 USPATFULL  
 TI Efficient method of synthesizing combretastatin A-4 prodrugs  
 AN 2002:221808 USPATFULL  
 TI Efficient method of synthesizing combretastatin A-4 prodrugs  
 IN Seyedi, Faye, Canton, MA, UNITED STATES  
 Gale, Jonathan, W. Townsend, MA, UNITED STATES  
 Haider, Reem, Lexington, MA, UNITED STATES  
 Hoare, John, Lunenburg, MA, UNITED STATES  
 Baldwin, Amy, Belmont, MA, UNITED STATES  
 PI US 2002119951 A1 20020829  
 AI US 2001-908321 A1 20010717 (9)  
 PRAI US 2000-218766P 20000717 (60)  
 DT Utility  
 FS APPLICATION  
 LREP Christopher C. Dunham, c/o Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, NY, 10036  
 CLMN Number of Claims: 35  
 ECL Exemplary Claim: 1  
 DRWN 5 Drawing Page(s)  
 LN.CNT 879  
 CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
 AB Methods of synthesizing a phosphate ester of combretastatin A-4 and trans-isomers thereof in which combretastatin A-4 is reacted with dibenzylphosphite in the presence of carbon tetrabromide, or with 2,2,2-trichloroethyl phosphorodichloridate, to form a phosphate ester of combretastatin A-4 with protecting groups thereon.  
 SUMM . . . Water-soluble prodrug derivatives of combretastatin A-4 have been reported recently. In particular, synthesis of phosphate salts of combretastatin A-4, designated "**combretastatin A-4P**" (Formula 2 below) have been found to impart the requisite water solubility to the prodrug and are disclosed in U.S. . . . Pat. No. 5,561,122 issued to G. R. Pettit et al. on Oct. 1, 1996. The phosphate group of the prodrug **combretastatin A-4P** reportedly is hydrolyzed in vivo to liberate the active drug combretastatin A-4. However, the currently disclosed methods for synthesizing **combretastatin A-4P** are difficult, require the use of undesirable solvents or restricted solvents, and are not easily scalable. ##STR2##  
 SUMM . . . of preparing prodrugs of combretastatin is necessary in order to meet the demand for an efficient and scalable synthesis to produce **combretastatin A-4P** and isomers thereof for effective use in treating cancer tumors and similar diseases.  
 SUMM [0012] As detailed herein, the subject invention provides a novel and improved method of synthesizing **combretastatin A-4P** that minimizes or eliminates the use of undesirable solvents, and overcomes many other deficiencies of the prior art using a. . .  
 DETD [0026] The difficulties with existing phosphorylation methods in the synthesis of **combretastatin A-4P** were investigated and a novel efficient synthesis of prodrugs of combretastatin A-4 was developed that substantially reduced the cost and time required to synthesize **combretastatin A-4P**. Table 1 summarizes the developments that were made to improve upon the current phosphorylation methods described above.

TABLE 1

Summary of.	Troc Phosphorylation Method
Entry	Improvements Result

1	Replacement of pyridine with triethylamine in phosphorylation	Reaction proceeded faster and gave white solid of <b>combretastatin A-4P</b>
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2 Replace DMF with Acetonitrile 71% crude yield  
Isolate intermediate Phosphate 46% recrystallization  
Acid of combretastatin A-4 98.3 wt % Assay

3. . . .

DETD . . . . for isolation. Deprotection of the intermediate is performed using acetonitrile in Zn/Cu amalgam to form the intermediate phosphate acid of **combretastatin A-4P**.

DETD . . . . to the Troc method disclosed in the prior art, resulting in a new and improved phosphorylation method to synthesize the **combretastatin A-4P** using Troc as a protecting group to form 3'-O-Bis-2,2, 2-(trichlorethyl) phosphate combretastatin A-4 (5). See Formula 4. ##STR4##

DETD . . . . and reagents such as chloroform, chlorotrimethylsilane/sodium iodide, and iodotrimethylsilane, which leave impurities that catalyze the conversion of cis isomers of **combretastatin A-4P** to the trans isomer resulting in product that is not optically pure. Further, these undesirable solvents and reagents are highly. . . .

DETD [0034] Synthesis of **combretastatin A-4P** was further improved using dibenzyl phosphite/carbon tetrabromide to phosphorylate the phenol combretastatin A-4 (Formula 1) with benzyl protecting groups thereon. . . .

DETD . . . . filtered out (Entry 7) in approximately 75% yield from cis-combretastatin A-4. In experimental results, the reported w/w assay of the **combretastatin A-4P** product was 81.4% desired (Entry 8). Since no impurities were observed in .sup.1H NMR and HPLC it was concluded that. . . .

DETD [0039] In order to remove impurities, crude **combretastatin A-4P** may be stirred into water/methanol mixture and the solution basified to pH 10-12 resulting in the crude product to become. . . .

DETD . . . . in solvent volume to gram of material are described in Table 3. Optimal results were obtained by recrystallizing the crude **combretastatin A-4P** material ("Product") with a mixture of water/methanol/acetone (5/5/10 ml/g crude) yielding in 40% recovery from starting retastatin A-4 (Entry 4).

TABLE 3

# Summary of Purification Methods

Entry	Improvements	Result
1	Recrystallization of <b>combretastatin A-4P</b> 3.6%, Recovery water/methanol/ acetone (mL/g solid) 4/4/8	wt/wt 97.2%, pH 7.98, Na 16%, KF 48%
2	Trituration of CA-4P 10% H.sub.2O/Acetone. . . .	wt/wt 98.1%, pH 7.59, Na
DETD	. . . . temperature for 30 minutes and filtered with an ethanol rinse (50 ml). In order to purify the product, the crude <b>combretastatin A-4P</b> (2.42 g) was dissolved in ml H.sub.2O Methanol 50% (24 ml) and the solution was filtered to remove any undissolved. . . .	
DETD	[0067] Crude <b>combretastatin A-4P</b> was isolated in approximately 75% yield (85% w/w assay). In order to purify the product, the crude <b>combretastatin A-4P</b> (260 g) was suspended in H.sub.2O (1300 ml) . Material dissolved as pH was adjusted to 10-12, using sodium. . . . ml) twice and then with acetone (445 ml). The isolated solid was dried in high-vacuum oven overnight at 40.degree. C. <b>Combretastatin A-4P</b> was isolated in 40% total yield from starting phenol.	
DETD	[0073] It can be appreciated that other salt forms of <b>combretastatin A-4P</b> may be formed by	